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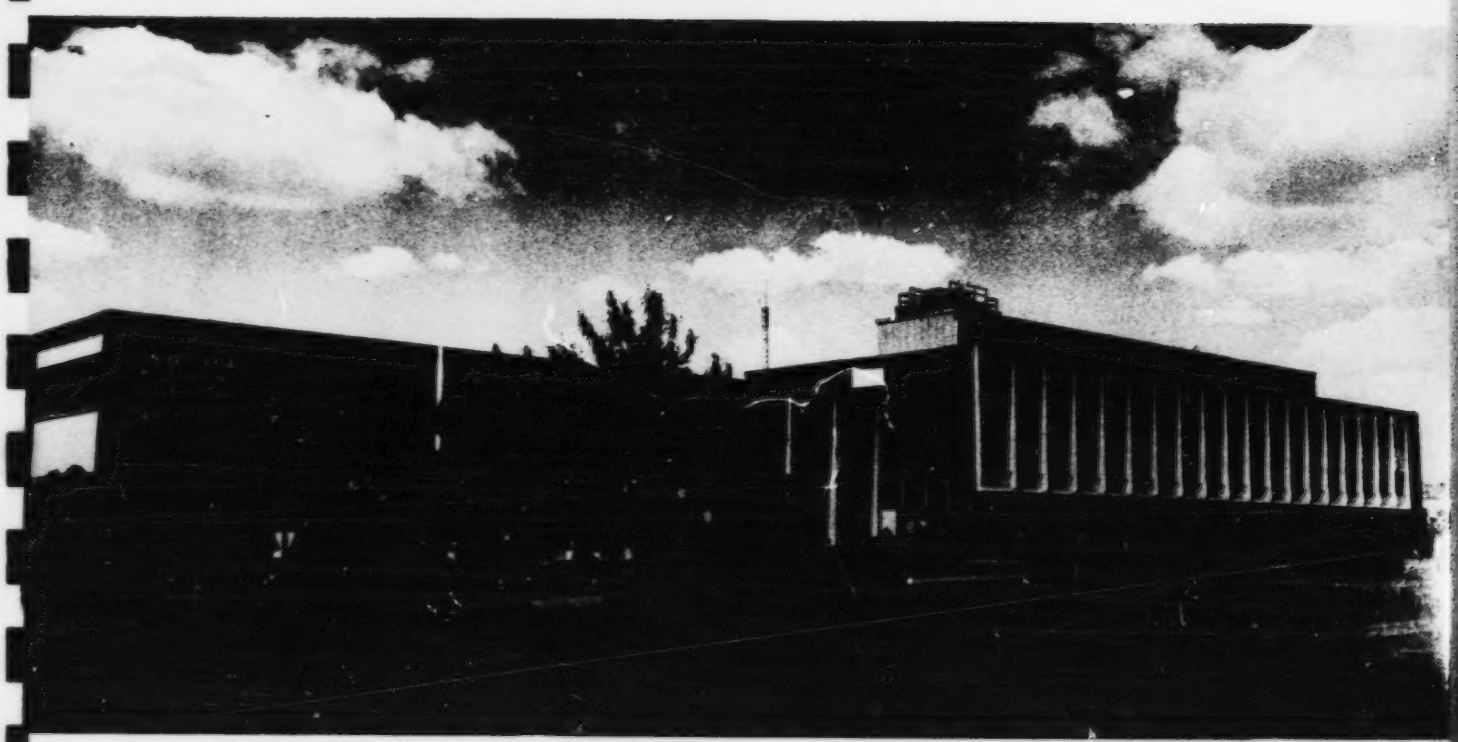

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OXIDATION OF ELEMENTAL TRITIUM BY ONTARIO SURFACE SOILS AND THE INFLUENCE OF SOIL MOISTURE AND TEMPERATURE ON RATES OF CONVERSION

Report No 82-525-K

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ABSTRACT

Soils representative of woodlot, pasture and cultivated land from the vicinity of Pickering and Bruce nuclear power developments were capable of oxidizing elemental tritium (HT, T_2) under laboratory conditions. When temperature was optimal (30°C), maximum oxidation activity of 104% conversion per hour occurred at a soil water content of 23% (w/w). In soil with a water content of 4% (w/w), the oxidation rate was three quarters of this maximal value but about 80% of this activity occurred by a non-biological process. At higher moisture levels oxidation was primarily biologically mediated. The rate of oxidation decreased as water content of soil approached field capacity (42%, w/w) and above field capacity remained constant at approximately one quarter of the maximal rate. Tritium oxidation in soil maintained at a water content of 125% (w/w) occurred at all temperatures tested with maximum conversion ($\sim$25%·h^{-1}) at 20 to 30°C. As temperature approached 50°C oxidation by non-biological means became increasingly important accounting for half of the tritium converted at 51.5°C. The presence of oxygen and reduction of free nitrogen in the atmosphere containing elemental tritium suggests that oxidation was, to some extent, mediated by aerobic, non-symbiotic nitrogen fixing bacteria.

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To Mr. F.J. Kee
Director of Research

OXIDATION OF ELEMENTAL TRITIUM BY ONTARIO SURFACE SOILS AND THE INFLUENCE OF SOIL MOISTURE AND TEMPERATURE ON RATES OF CONVERSION

INTRODUCTION

Tritium, a radionuclide released from nuclear power reactors is a fission product and activation product. Neutron activation is of particular importance in CANDU reactors which use heavy water (D_2O) in moderator cooling systems. Tritiated water (HTO) is released to the aquatic environment or to the atmosphere in vapour form. Emission of tritium gas (HT, T_2) will become increasingly important, however, with expanded use of nuclear generating facilities including reprocessing or heavy water upgrading plants and potential realization of fusion technology.

In the natural environment tritium behaves in a similar manner to hydrogen. Tritium gas (HT, T_2) is not readily retained by the body because of its low solubility and relative stability but tritium oxide (HTO) may be absorbed by inhalation and ingestion (McFarlane et al 1978). The radiobiological hazard resulting from tritiated water is therefore considerably higher than for an equal concentration of tritium gas (Garland and Cox 1980; McFarlane et al 1978). Correspondingly, permissible concentrations for occupational exposure to tritium gas may be up to 10,000 times higher than for tritium oxide (Sweet and Murphy 1981; Easterly and Phillips 1980).

Recent evidence from field (Watts and Murphy 1978) and laboratory (McFarlane 1978) studies has shown, however, that atmospheric contamination with tritium gas resulted in the rapid accumulation and persistence of tritiated water in vegetation. The site of tritium oxidation (HT, T_2 → HTO) was subsequently found to be the soil and the conversion process was shown to be biologically mediated (McFarlane et al 1978). Additional laboratory studies demonstrated that soils from several locations in the United States all had the capacity to oxidize elemental tritium which could be attributed to the presence of ubiquitous soil microorganisms (McFarlane et al 1978, 1980; Fallon 1982).

Rapid deposition and oxidation rates have also been demonstrated for in situ field studies with both gaseous tritium (Garland and Cox 1980; Sweet and Murphy 1981) and hydrogen (Conrad and Seiler 1981). Soil, particularly surface soil, was identified as the primary sink for elemental tritium while vegetation or water were relatively unimportant. Furthermore, deposition and oxidation were highest in soils of relatively low moisture content (Ibidem; Fallon 1982). Rates of oxidation were temperature dependent with maximum hydrogen uptake occurring at approximately 30°C (Conrad and Seiler 1981) while tritium uptake has been found to be highest at temperatures of 35 to 55°C (Fallon 1982).

The biological oxidation of elemental tritium has therefore been shown to represent a significant route of entry of tritiated water into the environment. Oxidized tritium may be taken up by flora and fauna from the surface water supply and subsequently incorporated into organic material (Garland 1979; Miettinen 1979; Garland and Cox 1980; McFarlane et al 1978). Garland and Cox (1980) estimated that the local dose resulting from T_2 to an individual living in southern Britain may be one eighth that derived from an equal release of HTO vapour. Considering that T_2 is deposited to land while HTO is deposited to land and sea, and allowing for the photochemical oxidation of elemental tritium in the atmosphere, they also estimated that the global dose of HT/T_2 may be 1.7 times that for HTO.

The recent awareness regarding the importance of elemental tritium oxidation and the potential input to regional tritium inventories from CANDU nuclear facilities prompted the present study. The objectives of this study were to determine the capacity of different soil biotopes in the vicinity of Ontario Hydro's nuclear generating stations to oxidize elemental tritium and to characterize the influence of soil moisture content and temperature on rates of oxidation.

MATERIALS AND METHODS

1. Description of Sample Locations

Soil samples representative of woodlots, cultivated land and pasture grassland were collected within 3 km of the Pickering and Bruce nuclear power developments. Both complexes are located in the Eastern Hardwood Forest region with the predominant great soil group of the area being Grey Brown Podzolic. Sample locations near Pickering consisted of a stonefree loam soil from a level or slightly undulating topography. A low to moderate stone phase in a loam texture and a gently rolling topography encompassed the sites sampled near the Bruce complex.

The collection sites at Pickering were located north-east of the station, just west of Duffins Creek (Figure 1). The woodlot was a mixed deciduous forest consisting mainly of maple with a few coniferous trees interspersed throughout the tree stand. A mature stand of corn was present at the cultivated soil location. The hard-packed soil from the pasture grassland was covered with a dense growth of long grass. All three sites were sampled on November 5, 1981. Additional grassland soil was collected from the same immediate location on June 14, 1982.

Soil samples from the Bruce area were collected on November 10, 1981 at a location north-east of the station (Figure 1). The woodlot site was a coniferous tree stand consisting mainly of cedar, although scattered deciduous trees were also present. At the cultivated site corn had been harvested prior to sampling. A pasture consisting of grass cover with rock outcroppings was also sampled.

2. Sample Collection

The top 0 to 13 cm of soil was collected using a flat shovel after removal of vegetation and litter from the sampling areas. Large pieces of organic debris and stones were removed from the soil samples with the remaining, well mixed soil, being stored in tightly sealed opaque polyethylene bags at room temperature (20°C; relative humidity 70%). Additionally, 2.5 cm^2 blocks of intact soil were taken for subsequent determinations of water content at field capacity. These samples, tightly wrapped with cellulose acetate and placed in plastic bags to exclude air, were stored at a temperature of 22°C and relative humidity of approximately 90% to lessen evaporation.

The capacity for tritium oxidation was also investigated for new and used potting soil, an artificial soil mixture (Redi-Earth) prepared by W.R. Grace and Co. The used potting soil had been previously employed for propagation of tomatoes.

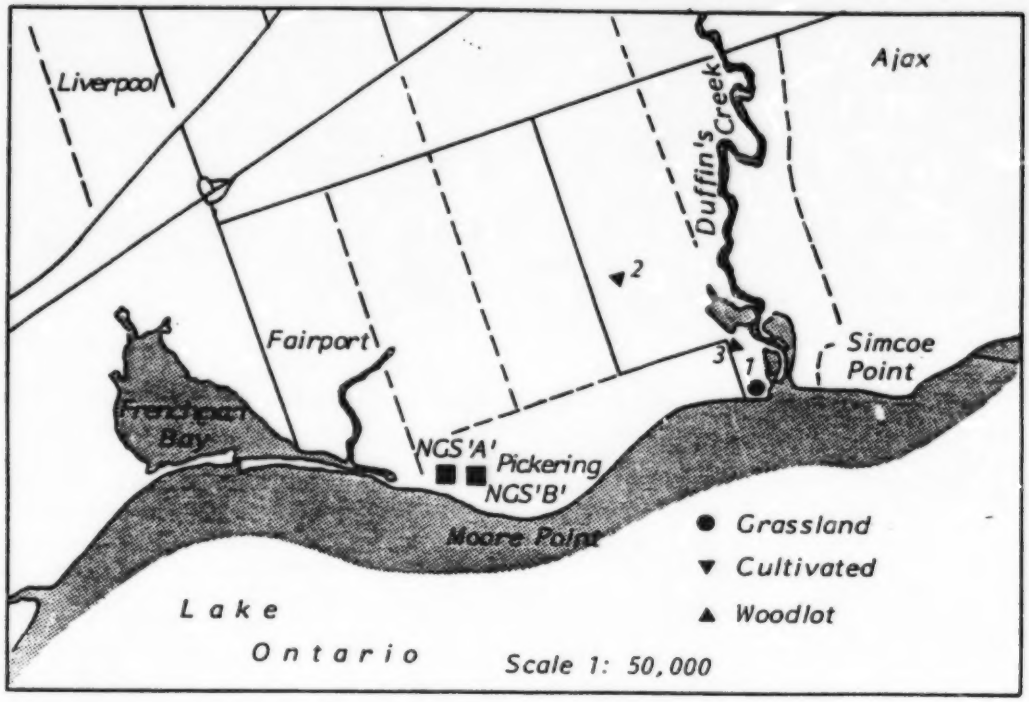
3. Soil Characteristics

3.1 Physical and Chemical Analysis

Soil samples from the six field locations were sent to the Ontario Ministry of Agriculture and Food soil testing laboratory operated by the University of Guelph for the analysis of phosphorus, potassium, calcium, magnesium, pH, % total nitrogen, % organic matter and the determination of soil texture (particle composition).

3.2 Water Content

Water content (WC) expressed as the weight of water (g) per gram of dry soil was determined following the drying of soil samples at 70°C for 24 h.



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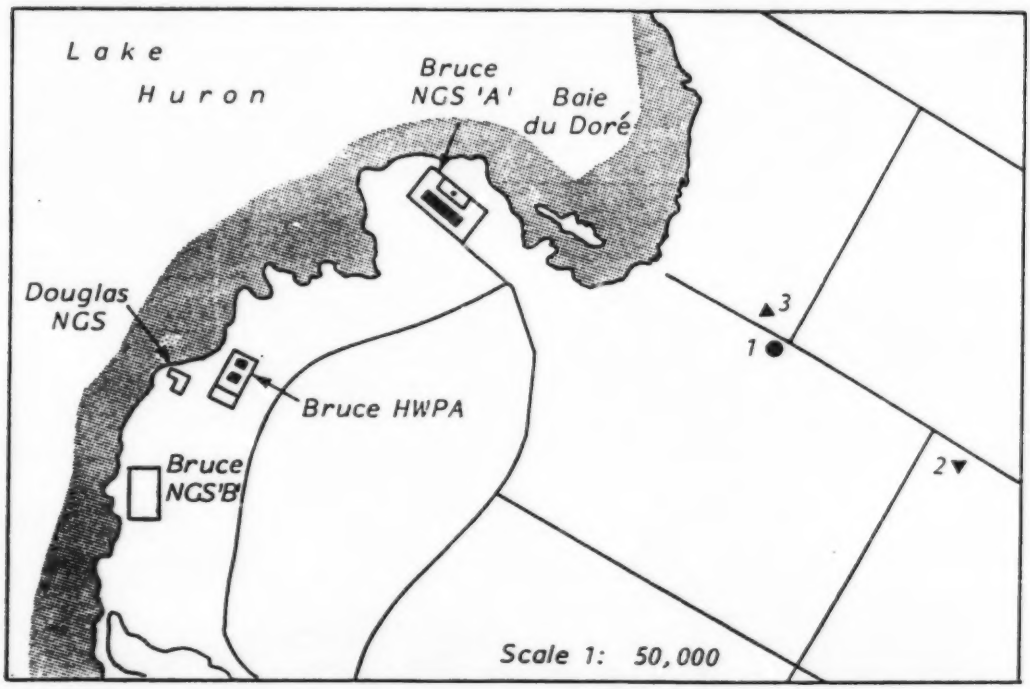


(b)

FIGURE 1

SAMPLE LOCATIONS FOR SOILS USED IN TRITUIM OXIDATION EXPERIMENTS

$$WC = \frac{\text{Soil Wet Weight - Soil Dry Weight}}{\text{Soil Dry Weight}} \quad (1)$$

Initial determinations indicated that weight loss during drying had stabilized by 24 h (Appendix I). Although samples were dried at 70°C to prevent loss of organic matter, limited tests indicated that an additional 24 h drying period at 105°C did not result in increased weight loss.

Two standard measures of soil water content, field capacity and water holding capacity, were determined. Both terms have appeared in literature relevant to the present study and have therefore been included to enable comparison of results. Field capacity represents the maximum amount of water that can be held against the force of gravity and is a measure of the maximum moisture content that a soil can maintain for an extended period. The maximum water holding capacity (WHC), characteristically the larger of the two values, is the moisture content of a 1-inch (2.54 cm) layer of loose soil after gravitational water has drained from the largest pores (Fink 1960).

An adaptation of the suction plate method described by Radhakrishna (1968) was used to determine field capacity. Duplicate cores of soil were taken by pressing a brass cylinder with a depth of 2.54 cm and diameter of 2.86 cm into the intact soil samples. Weight of the sample was obtained by subtracting the weight of the cylinder. Samples were pressed onto filter paper overlying a porous ceramic stone (1 bar) and were water saturated. The samples were covered with a damp cloth and plastic wrap to prevent evaporation and allowed to drain under 45.7 cm (0.05 atmospheres) of capillary tension. Weights were taken daily until no further weight loss occurred at which time samples were dried at 70°C for 24 h. Water content of the soil at field capacity was determined from the drained and dried soil weights (equation 1).

Water holding capacity was determined (in triplicate) by loosely packing soil to a depth of 2.54 cm on filter paper lining the bottom of a 5.08 cm diameter plastic pot. After soaking samples in a pan of water to saturation (24 h) they were removed and allowed to drain for 5 h (Klute 1965). The weight difference between drained and dried soil was used to calculate water content (equation 1) at WHC.

4. Experimental

4.1 Soil Comparisons

Procedures followed in comparison of tritium oxidation rates for the eight soil samples were adopted from McFarlane et al (1978, 1979).

One week prior to experimentation the moisture content of the stored soil samples was determined. Sufficient soil (540 g, dry weight basis) for experimenation was adjusted to 50% WHC and incubated for 7 days at 30°C in covered beakers. Water was added daily (except weekends) to maintain constant moisture levels.

Following incubation, moist soil (20 g, dry weight basis) was placed in 1 L round bottom flasks (24/40 neck) and enough water added to bring the samples to 140% WHC. The flasks were closed with rubber stoppers and the soil slurry was swirled over the inner surface of the flask. An air volume of 1.5 cc was removed by syringe from the flask and an equal volume of elemental tritium (1.65 μCi T_2) in a nitrogen carrier was injected into the flask. Needle punctures in the stopper were immediately sealed with stop-cock grease and the entire top of the flasks covered with Parafilm. Flasks were then incubated at 30°C. Triplicate soil samples were removed at times of 0, 1, 2, 8, 20, 26 and 48 h following tritium injection. Stoppers were removed, the flasks were flushed with air and 50 mL of toluene added to arrest further oxidation. Distillation in a toluene/water azeotroph was carried out in the same flask and tritium, recovered as water, was determined in a liquid scintillation counter (sample volume to PCS II cocktail volume = 1:14).

Sub-samples of soil incubated for 7 days were autoclaved following transfer to the 1 L flasks and adjustment to 140% WHC. These samples served to identify levels of conversion attributable to abiotic factors and were treated as described above except that analysis were conducted at 0 and 48 h only. Both biological and abiological tritium counts were corrected for background tritium activity, determined using aquifer water and for instrument counting efficiency (cpm → dpm).

4.2 Influence of Soil Moisture Content

The effect of soil water content on the rate of tritium oxidation was examined by adjusting the moisture level of Pickering grassland soil to 5, 25, 50, 75, 100 and 140% WHC following the seven day adaptation period. Samples were incubated at 30°C. The experimental procedure was the same as described above with a few minor revisions. The time-course of tritium oxidation in previous experiments (soil comparisons) indicated a 4 h sample to be preferable to one taken at 20 h. Also, the initial unsuccessful attempt to estimate the rate of tritium conversion at 25% WHC indicated the necessity of more frequent sampling immediately following tritium addition (0 to 4 h). Low water content of soil adjusted to 5 and 25% WHC necessitated the addition of supplemental water (10 and 5 mL respectively) to those samples immediately following fixation with toluene and prior to distillation.

A replacement in the tritium supply resulted in two levels of elemental tritium, 1.65 and 9.75 μCi, being used in different WHC experiments (Appendix II). Although this was not expected to affect estimated oxidation rates, verification was desired and thus rates of oxidation for soils at 50 and 75% WHC were determined based on the two different tritium levels.

4.3 Influence of Soil Temperature

The effect of incubation temperature on the rate of tritium oxidation was determined, again using the Pickering pasture-grassland soil. Temperatures of 0, 10, 20, 30, 40, 50 and 51.5°C were tested for soil maintained at 140% WHC.

4.4 Gas Analysis

Gas chromatography was used to determine the CO_2, CO, N_2 and O_2 content in supplemental flasks of Bruce grassland soil (30°C, 140% WHC) at 0 h and 48 h following tritium injection. Analyses were performed by the Analytical Services Section of the Research Division.

5. Data Analysis

The time course of tritium oxidation was fitted to a curve described by the exponential growth model (McFarlane et al 1979, 1980):

$$C_t = C_\infty [1-\exp(-kt)] + E \qquad (2)$$

where C_t is the amount of tritium oxidized in time t, C_∞ is the total amount of tritium oxidized (asymptotic value), k is the reaction rate parameter (h^{-1}), t is the time in hours and E is the error function.

The instantaneous change in the amount of tritium oxidized:

$$\frac{dC_t}{dt} = k \cdot C_\infty e^{-kt} \qquad (3)$$

is maximal when $t = 0$, being equal to $k \cdot C_\infty$. If the amount of oxidized tritium is measured as a percentage of total elemental tritium injected, then the asymptote C_∞ equals 100% (assuming complete oxidation) and k equals the maximum conversion rate, or the "initial" velocity of the reaction (per cent oxidation per hour). As time progresses, however, the concentration of elemental tritium declines and so, therefore, does the rate of conversion.

McFarlane et al (1979) assumed the errors associated with the measurement of tritium oxidation to be independent and to come from a normal Gaussian distribution. Examination of data in the present study indicated that the normal distribution assumption was appropriate although

(1) replicates at t = 0 had a much smaller variance than replicates at other times with the variances of replicates at t > 0 being similar within a soil type, and

(2) the variances of different soil types were similar except for Pickering grassland soil (Schwarz 1982).

A non-linear least squares procedure was used to fit experimental results to the model. Parameter estimates for C_∞, k and associated variances were determined by an iterative technique. It should be noted that the derived 95% confidence intervals are only approximate since the model is non-linear in its parameters.

RESULTS

1. Comparison of Soil Types

Typical curves depicting the time course of tritium oxidation are presented in Figure 2 for the Pickering woodlot and used potting soil samples which represented the highest and lowest mean oxidation rates respectively. Similar curves for other soil samples together with non-linear least squares analysis and residual plots of the data indicated that, with the exception of the Pickering grassland sample, the model was highly representative of the data (Appendices III, IV).

Mean oxidation rates expressed as per cent conversion (T_2, HT → HTO) per hour for the six field collected samples ranged from 14.7% (Pickering cultivated) to 27.8% (Pickering woodlot) (Table 1). Oxidation rates of the three Bruce soils ranged from 18.3 to 23.8% per hour. The 95% confidence intervals for the Pickering grassland sample which encompassed the oxidation rates of all other Pickering and Bruce samples reflected the high degree of replicate variability observed in this data set, particularly at the 8 h sample period. The variances associated with other soil samples were much lower (Appendix V).

Oxidation of elemental tritium in all eight soil samples was primarily a biologically mediated process. The amount of tritium converted to HTO in heat sterilized samples after 48 h incubation was, in all cases, less than 4% of the tritium converted in soil samples possessing an active microbial population (Table 1).

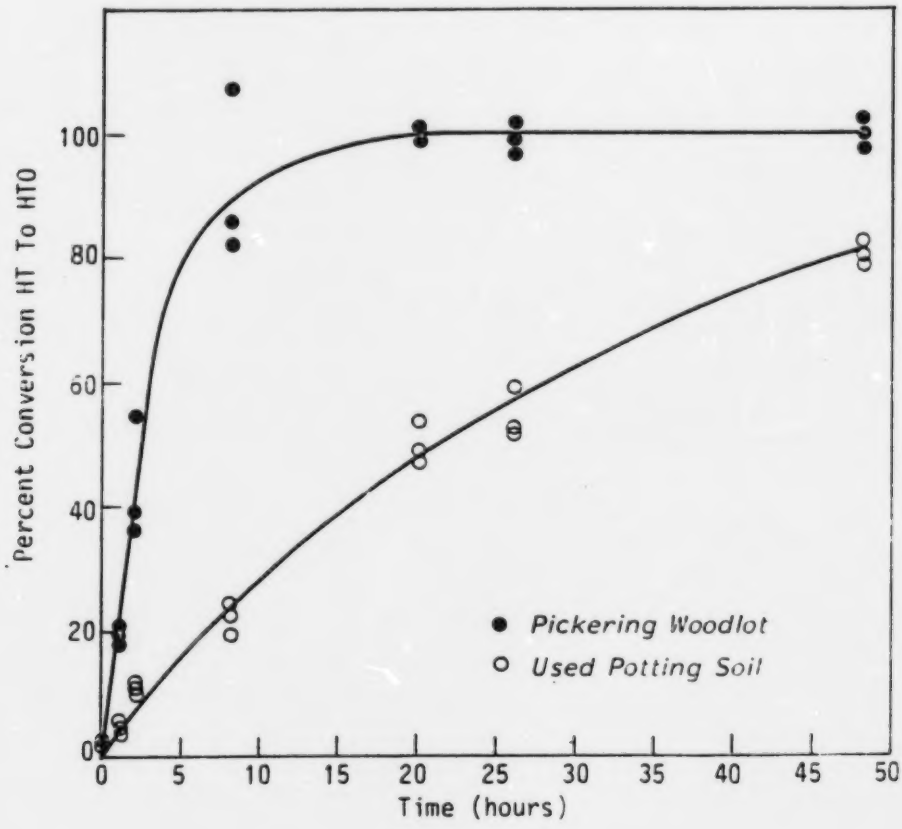


FIGURE 2

TIME COURSE OF ELEMENTAL TRITIUM OXIDATION IN SAMPLES OF PICKERING WOODLOT SOIL AND USED POTTING SOIL

TABLE 1

MEAN RATES OF TRITIUM OXIDATION AND 95% CONFIDENCE INTERVALS (CI) FOR EIGHT SOIL TYPES AND PER CENT MICROBIAL OXIDATION AFTER 48 HOURS OF INCUBATION (TEMP = 30°C; WATER CONTENT = 140% WHC)

Location/ Soil Type	Lower 95% CI Bound for Oxidation Rate ($\%.h^{-1}$)	Estimate of Oxidation Rate Per cent Per Hour	Upper 95% CI Bound for Oxidation Rate ($\%.h^{-1}$)	Microbial Oxidation as a Percentage of Total Oxidation
Pickering Woodlot	22.7	27.8	32.9	> 99
Pickering Cultivated	12.7	14.7	16.6	> 99
Pickering Grassland	8.5	20.9	33.3	98
Bruce Woodlot	17.9	20.0	22.0	97
Bruce Cultivated	18.1	23.8	29.4	98
Bruce Grassland	15.6	18.3	20.9	99
New Potting Soil	4.7	5.2	5.8	> 99
Used Potting Soil	2.5	3.3	4.0	98

The six field-collected soil samples were similar in physical composition with clay content ranging from 22 to 35%, silt content from 34 to 58% and sand content from 15 to 34% (Table 2). Soil texture was not related to the biotopes tested: woodlot, pasture-grassland, cultivated.

The oxidation rates measured for the six soil types were inadequate to enable derivation of empirical relationships between oxidation rates and the physical or chemical composition of the soils. Some general observations of a suggestive nature were, however, apparent. Although the phosphorus content of the Pickering soils was several times higher than in the Bruce soils, this had no apparent effect on the rates of oxidation. Similarly no relationship to magnesium or potassium content of the soils was indicated.

The pH of the soils ranged from 7.0 to 7.7 and the organic content from 4.3 to 17.6% (Table 2). A curvilinear relationship existed between organic content and total nitrogen content of the soils. The highest oxidation rate occurred in soil having the lowest pH and highest organic (total N) content (Pickering woodlot) while the lowest oxidation rates were recorded for Pickering cultivated and Bruce grassland soils which had the highest pH values and lowest organic content.

In comparison to the field-collected soil samples the oxidation rate of both new and used potting soil was much lower with a conversion efficiency of 3 to 6% per hour (Table 1). One major difference between potting soil and the natural soils which may have in part accounted for the differences in oxidation rates was water content. At water holding capacity the six field-collected samples contained from 0.57 to 1.29 g water per gram soil while the potting soil contained 7.41 g water per gram soil (Table 3). The moisture content of the soils at field capacity was 46 to 56% of the water holding capacity values. The depression of tritium oxidation rate as a function of soil water content is discussed in the section following.

Change in gaseous content of the sample flasks over the 48 h incubation period was determined for the Bruce grassland soil (Table 4). Gaseous nitrogen content of the flasks was reduced to approximately 25% of the initial concentration after 48 h indicating the occurrence of nitrogen fixation during incubation. There was a six-fold increase in carbon dioxide concentration during incubation although no corresponding decrease in oxygen concentration occurred as would be expected to result from respiration activity. Carbon monoxide levels were below the detection limit of 5 µL/L at both 0 and 48 h.

TABLE 2

PHYSICAL AND CHEMICAL COMPOSITION OF PICKERING AND BRUCE SOIL SAMPLES

Location	Phosphorous (ppm)	Potassium (ppm)	pH	Calcium (ppm)	Magnesium (ppm)	% Total Nitrogen	% Organic Matter	Texture	Particle Composition		
									% Clay <2 µm	% Silt 2 to 53 µm	% Sand 50 to 2000 µm
Pickering Woodlot	33	112	7.0	2000	200	0.50	13.2	Clay loam	28	44	27
Pickering Cultivated	27	132	7.7	2000	124	0.20	4.3	Silty clay loam	28	58	15
Pickering Grasslands	50	248	7.4	2000	156	0.41	8.5	Clay loam	32	34	34
Bruce Woodlot	5	88	7.7	2000	200	0.56	17.6	Loam	22	47	32
Bruce Cultivated	11	184	7.5	2000	200	0.33	6.5	Clay loam	35	41	24
Bruce Grasslands	2	164	7.6	2000	200	0.21	4.3	Silty clay loam	31	54	15

TABLE 3

MOISTURE CONTENT OF SOILS AT WATER HOLDING CAPACITY (WHC) AND FIELD CAPACITY (FC)

Soil	Location	g water per g soil 100% WHC	100% FC	FC/WHC
Woodlot	Pickering	1.0176	0.5011	0.49
Cultivated	Pickering	0.5695	0.2620	0.46
Grassland	Pickering	0.8934	0.4176	0.47
Woodlot	Bruce	1.2914	0.6941	0.54
Cultivated	Bruce	0.6546	0.3325	0.51
Grassland	Bruce	0.5697	0.3178	0.56
Potting	-	7.4140	3.5627	0.48

TABLE 4

GASEOUS CONTENT OF SAMPLE FLASKS CONTAINING BRUCE GRASSLAND SOIL BEFORE AND AFTER 48 HOURS INCUBATION

Gas	Concentration (mL/L)	
	Initial	48 h
Nitrogen	790	208
Oxygen	208	208
Carbon Dioxide	0.560	3.320
Carbon Monoxide	<0.005	<0.005

2. Influence of Soil Moisture Content

The greatest rate of tritium oxidation (104% per hour) occurred at a soil water content equivalent to 25% WHC (Figure 3). A doubling of the soil water content (50% WHC) reduced the rate of oxidation to approximately one third of the rate occurring at 25% WHC. At still higher water contents (75-140% WHC) rates were consistent, generally being less than one quarter of the maximal value observed at 25% WHC. The oxidation rate in very dry soil (5% WHC) was the second highest value observed. Examination of the relative contribution of microbial and abiotic (chemical and/or physical) processes to total oxidation after 48 h incubation revealed that biological activity accounted for only 19% of the total tritium converted in samples maintained at 5% WHC. In contrast, microbial oxidation accounted for over 99% of tritium conversion in all other samples (Figure 3B). The soil for the 5% WHC experiment (Pickering grassland), although collected from the same location as soil used in other water content experiments, was collected at a later date. In view of the consistently low levels of abiotic oxidation observed at 30°C for different soils (Table 1), it seems unlikely that the date of collection would have had such an extraordinary influence on tritium conversion by abiotic processes.

The seven day adaptation period at 50% WHC and 30°C was assumed to have resulted in a uniform microbial composition and stable level of biological activity in all experiments involving Pickering grassland soil. This was confirmed by the experiments conducted on three different occassions over a three month period under conditions of 140% WHC and 30°C. Oxidation rates in these samples were similar ranging from 20.9 to 23.6% conversion per hour (Figure 3A). This indicated that aging of soil in the laboratory had little influence on oxidation rate or on the relative contribution of microbial and abiotic processes to conversion efficiency. Following acclimation, samples maintained at 50% WHC or adjusted to water contents of 75 to 140% WHC were likely to have had similar microbial levels, at least at the start of the experiments. Samples adjusted to a WHC of 5 and 25% required air-drying for periods of 24 and 7 hours respectively which may have resulted in a change in microbial biomass.

The apparent relationship between oxidation rate and soil water content may have also been influenced by differences in aerial exposure of the soils. At WHC of 100 and 140% a soil slurry was formed which covered the entire inner surface of the flask. A patchy coverage of the flask surface occurred in samples with lower water content (50 and 75% WHC). At 5 and 25% WHC soil did not adhere to the surface of the flasks but rather settled to the bottom. However, interstitial air space in the soil could be expected to increase with a reduction in water content. The extremely low

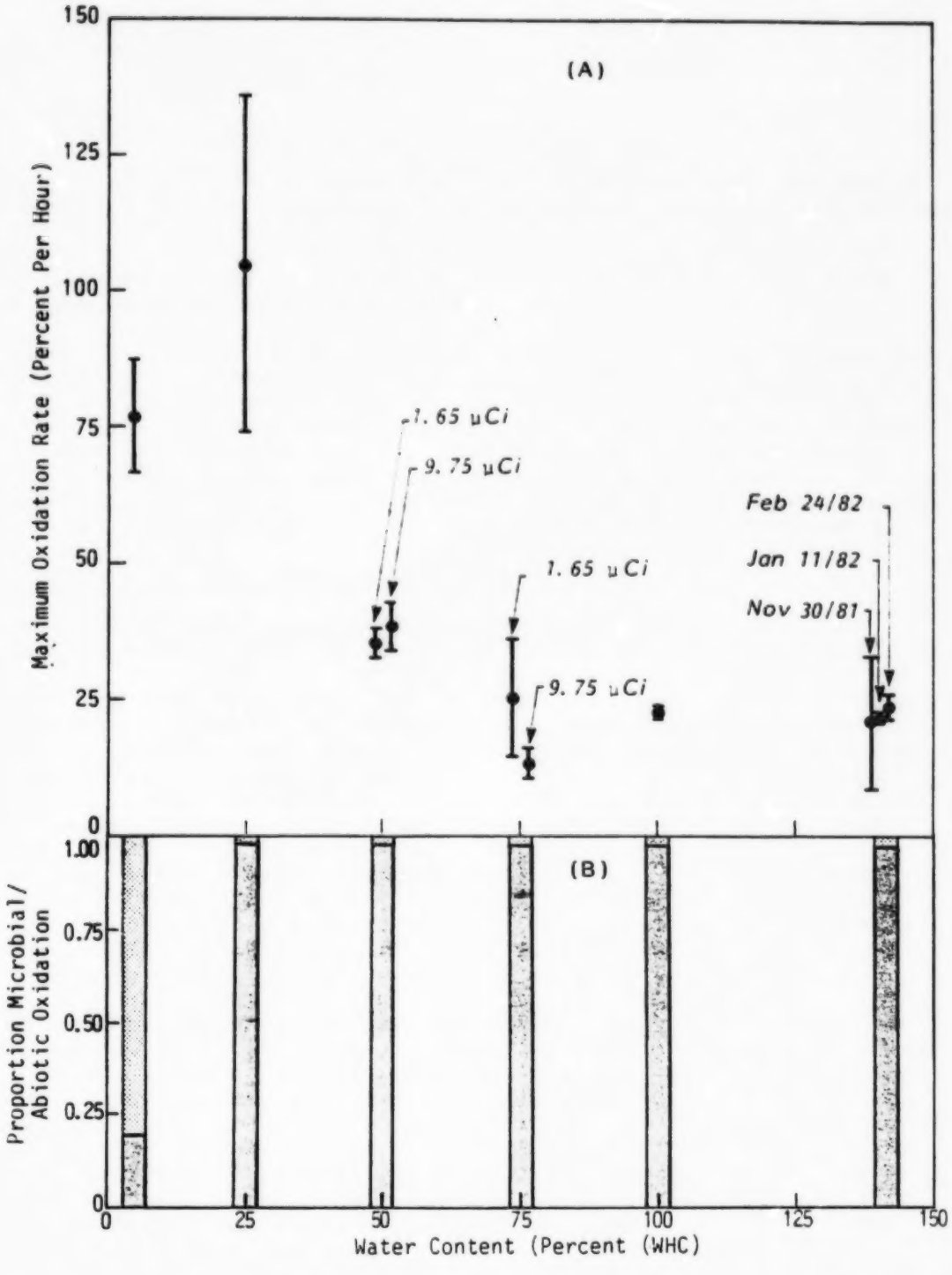


FIGURE 3

OXIDATION OF ELEMENTAL TRITIUM IN RELATIONSHIP TO SOIL WATER CONTENT SHOWING:
(A) MEAN OXIDATION RATES (PERCENT CONVERSION PER HOUR) AND 95% CONFIDENCE INTERVALS, AND
(B) RELATIVE CONTRIBUTION OF MICROBIAL (SOLID) AND ABIOTIC (STIPPLED) OXIDATION PROCESSES AFTER 48 HOUR INCUBATION.
PICKERING PASTURE - GRASSLAND SOIL,
- INCUBATION TEMPERATURE - 30°C

solubility of tritium in water (0.0005%) indicates that the surface reactive area of the soil would be much greater under arid conditions thereby accounting for the higher oxidation rate in soils with a reduced water content. At a WHC of 75% or greater oxidation was likely limited to the soil surface since interstitial soil space would have been saturated with water.

Estimates of tritium oxidation rates were assumed to be independent of the amount of elemental tritium introduced to the flasks (1.65 and 9.75 µCi) since conversion occurs by a first order reaction (equation 2). Rates of oxidation determined from the two tritium levels were nearly identical for soil adjusted to 50% WHC while in soil adjusted to 75% WHC a higher mean oxidation rate was indicated for an initial tritium level of 1.65 µCi although the 95% confidence intervals for the two estimates overlapped (Figure 3A).

3. Influence of Soil Temperature

Greatest oxidation of tritium in soils maintained at 140% WHC occurred at temperatures of 20 and 30°C with no clear preference as a result of the broad 95% confidence intervals at 20°C and in the November 30, 1981 experiment for 30°C (Figure 3A). As mentioned previously, mean oxidation rates at 30°C, determined on three separate occasions, were similar. Elemental tritium was oxidized over the entire temperature range tested (0 to 51.5°C) with mean conversion rates ranging from 9.9 to 26.8% per hour. Although a general trend between rate of oxidation and temperature existed between temperatures of 10 and 40°C this pattern did not persist at the lowest and highest temperatures tested. The lowest mean oxidation rate occurred at 0°C but deviated from the bell-shaped temperature curve most typical of biologically mediated processes. Rates of oxidation at 0 and 10°C were similar, being less than half the mean oxidation rate occurring at 20 and 30°C.

The rate of oxidation also declined at 40°C but then increased at a temperature of 51.5°C. Again, however, variability within these data, as suggested by the 95% confidence intervals, indicated that tritium conversion may not have been significantly different from the maximal rates occurring at 20 and 30°C. Approximately one half of the tritium converted ($20\%.h^{-1}$) after 48 h in the 51.5°C soil resulted from abiotic oxidation (Figure 4B) accounting for the increase in total tritium converted at this temperature. At 50°C abiotic oxidation was found to account for 13% of total tritium converted after 48 h incubation. Since at temperatures of 40°C or less over 99% of the elemental tritium was converted by microbial activity, a rapidly increasing contribution of physical and/or chemical processes to tritium oxidation occurred as the temperature approached 50°C.

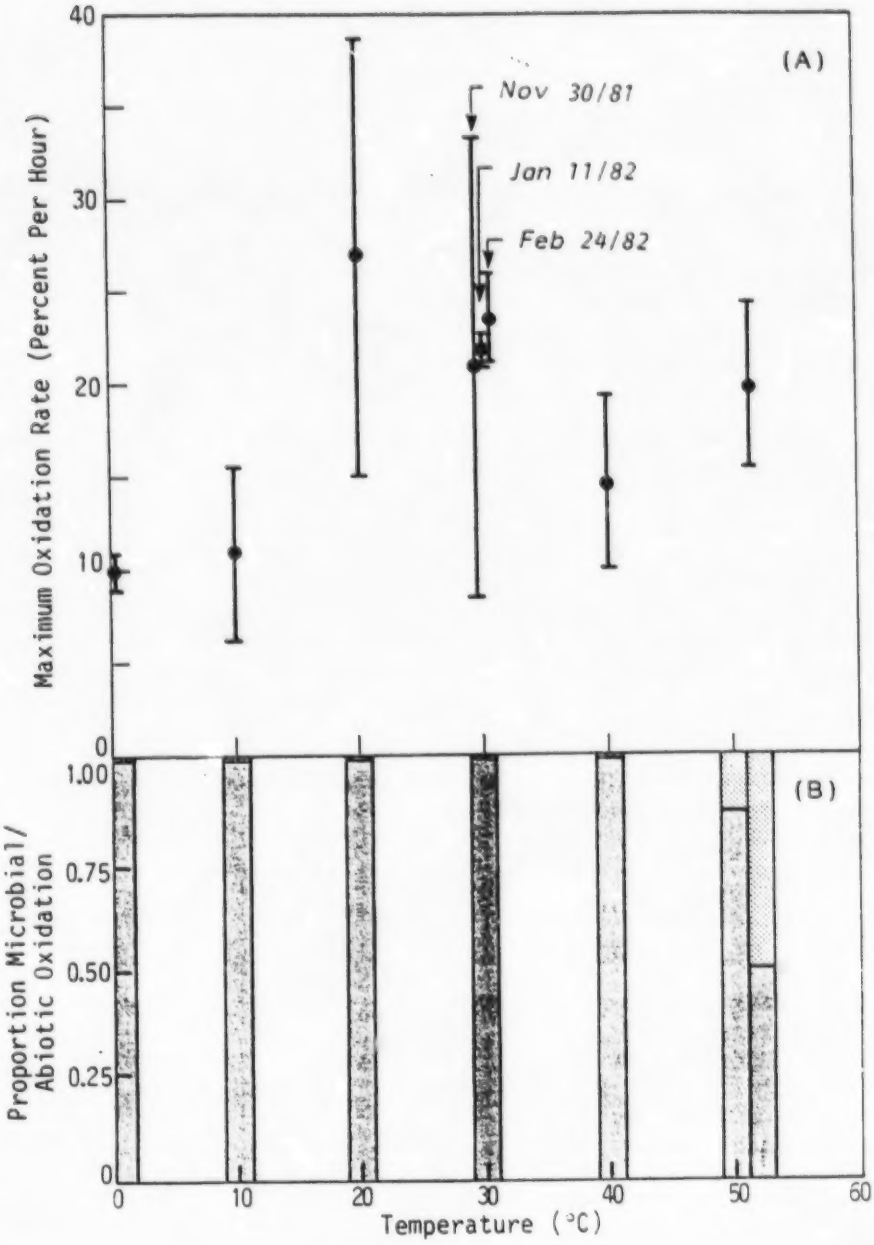


FIGURE 4

OXIDATION OF ELEMENTAL TRITIUM IN RELATIONSHIP TO SOIL TEMPERATURE SHOWING
(A) MEAN OXIDATION RATES (PERCENT CONVERSION PER HOUR) AND 95% CONFIDENCE INTERVALS, AND
(B) RELATIVE CONTRIBUTION OF MICROBIAL (SOLID) AND ABIOTIC (STIPPLED) OXIDATION PROCESSES AFTER 48 HOURS INCUBATION.
PICKERING PASTURE - GRASSLAND SOIL;
- WATER CONTENT - 140% WHC

DISCUSSION

Rates of tritium oxidation for six Ontario soils ranged from 15 to 28% conversion (T_2, HT → HTO) per hour and were within values of 12 to 66% reported for soils from several US locations by McFarlane et al (1978, 1979). The experimental procedure in both studies was the same. The present study, therefore, supports the contention that the capacity for tritium oxidation is a common property of soils and that soil is an important sink for the deposition of gaseous tritium present in the atmosphere.

Velocities of tritium deposition to soil calculated from the laboratory results of McFarlane et al (1978, 1979) ranged from 0.001 to 0.03 $cm \cdot s^{-1}$ (Sweet and Murphy 1981). While laboratory results may not be directly comparable to field conditions (Ogram, pers.comm.) studies have shown tritium uptake to proceed at a faster rate during field exposure of intact soils. Sweet and Murphy (1981) found *in situ* deposition velocities of approximately 0.03 $cm \cdot s^{-1}$ when wind speed within the experimental chamber was low and 0.13 $cm \cdot s^{-1}$ at higher wind speeds. *In situ* deposition velocities for grassland soil which ranged from 0.01 to 0.1 $cm \cdot s^{-1}$ were found to vary with season being highest in the drier summer/autumn period (Garland and Cox 1980). Deposition velocities determined in the laboratory using soil with a low moisture content were between 0.024 and 0.048 $cm \cdot s^{-1}$ (Fallon 1982). These values were comparable to field estimates of tritium or hydrogen uptake (Ibidem; Conrad and Seiler 1981).

A clear relationship has been found to exist between oxidation rate and moisture content of the soil. Tritium deposition is greatly reduced in soils with high moisture content such as the level used by McFarlane et al (1978, 1979) and in the soil comparisons of the present study (140% WHC; 125% w/w). This has been attributed to the inhibition of gaseous diffusion due to the low solubility of tritium (Sweet and Murphy 1981; Garland and Cox 1980; Fallon 1982). Maximum tritium uptake in sandy soil was reported in the range 1 to 10%(w/w)with maximum uptake in sandy loam or loam soils generally occurring at higher moisture levels (10 to 23%) (Fallon 1982; Conrad and Seiler 1981). With Pickering grassland soil maximum uptake occurred at a water content of 23% (w/w; equivalent to 25% WHC or = 54% FC) and at moisture levels equivalent to field capacity (= 45% w/w) tritium uptake was reduced to approximately one third of the maximal rate. Garland and Cox (1980) also noted that relatively dry soil absorbed tritium two to three times faster than soil wetted to field capacity. Fallon (1982) reported a broad region of constant high uptake activity but a marked decline in activity occurred when water content of the soil exceeded field capacity.

In very dry soils, with a water content of less than 1% (w/w), Conrad and Seiler (1981) and Fallon (1982) reported that no oxidation occurred and attributed this to a loss of enzyme activity. The large abiotic component (81%) contributing to tritium oxidation ($77\%.h^{-1}$) in relatively dry soil (4% w/w) in this study was of particular interest since oxidation had previously been attributed to biological processes even at low soil moisture contents.

A relationship between the rate of tritium uptake and the pH/organic content of the six Ontario soils was noted. This observation may have been coincidental, however, since McFarlane et al (1979) in a comparable study found tritium oxidation rates of 12 soils to be independent of soil type or chemical properties including pH (range 4.6 to 8.2%) and organic content (range 0.4 to 6.2%). Also existence of such a relationship was not apparent in field studies (Sweet and Murphy 1981; Garland and Cox 1980).

Temperature has been found to influence the rate of tritium oxidation, as would be expected of a biologically mediated process, although variations exist in the reported nature of this relationship. In the present study oxidation occurred over the entire temperature range tested (0 to 51.5°C) with maximum activity occurring at 20 to 30°C. Fallon (1982) reported oxidation to occur at temperatures of -20°C to 65°C with maximum activity between 35 and 55°C. In both studies tritium oxidation at 0°C was only slightly less than one-half of the maximal rates. Similarly, Sweet and Murphy (1981) reported tritium deposition and oxidation to be only slightly inhibited at 0°C. Hydrogen oxidation at temperatures of 3 and 45°C was found to be 50% of the maximal rate which occurred at 30°C in German soils (Liebel and Seiler 1976). Fallon (1982) suggested that the rapid tritium uptake observed at higher temperatures in the South Carolina soils he tested may have been due to biological adaptation which occurs in warmer environments. Garland and Cox (1980), however, suggested that seasonal changes in in situ tritium uptake rates were affected primarily by soil moisture levels. At the highest temperature tested in this study (51.5°C) a substantial portion (50%) of total oxidation was attributed to abiotic processes. This may account in part for sustained oxidation at the high temperatures reported by Fallon (1982).

Apart from soils maintained at high temperatures (> 50°C) or low soil moisture levels (<5% w/w) the importance of biological oxidation of elemental tritium (or hydrogen) has been amply demonstrated in this study and others (McFarlane et al 1978, 1979; Fallon 1982; Garland and Cox 1980; Sweet and Murphy 1981; Conrad and Seiler 1981). Consideration of the microorganisms or metabolic pathways responsible for this oxidation has, however, received little attention (Conrad and Seiler 1979, 1981; Fallon 1982).

Oxidation of hydrogen has been attributed to the enzyme hydrogenase which occurs in many microorganisms of wide taxonomic and metabolic diversity. A comprehensive review of bacterial hydrogenase has been compiled by Mortenson and Chen (1974). The reaction catalized by hydrogenase is

$$H_2 \rightleftarrows 2H^+ + 2e^- \qquad (4)$$

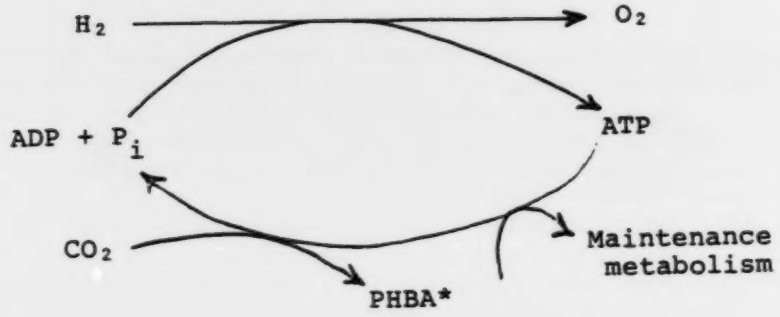
The electrons liberated during oxidation are transferred to a primary electron acceptor which serves as a source of energy in further reactions. Different primary electron acceptors have been identified (NAD^+, cytochrome c_3, ferredoxin) while other electron carriers and associated enzymes are involved in energy transfer (Krasna 1979; Repaske 1966).

In the present study the atmospheric content of oxygen in the Bruce grassland samples remained near ambient levels during the 48 h incubation period. This indicated that tritium oxidation occurred under aerobic conditions since no large gradient in oxygen tension was likely in the thin film of soil spread over the inner surface of the flasks. It is interesting to note that Conrad and Seiler (1981) found reduced hydrogen oxidation under anaerobic conditions but uptake remained constant at oxygen levels of 1 to 20%.

Under aerobic conditions two different metabolic groups of bacteria are of potential importance in the oxidation of elemental tritium. The first group consists of non-photosynthetic autotrophs known as the hydrogen or "knallgas" bacteria which are capable of growing autotrophically on CO_2 with H_2 serving as the sole energy source (Bowien and Schlegel 1981; Davis et al 1969). Although different electron acceptors may be involved in the oxidation of H_2, oxygen is the terminal oxidant under aerobic conditions (Mortenson and Chen 1974; Repaske 1966). The knallgas or oxyhydrogen reaction is

$$2\ H_2 + O_2 \rightarrow 2\ H_2O \qquad (5)$$

The hydrogen bacteria obtain energy for growth from conventional phosphorylation coupled to reduction of oxygen by electrons supplied by hydrogen (Repaske 1966). In the presence of CO_2 hydrogenase activity is maximal since synthesis leading to growth (CO_2 serving as a carbon supply) is energy requiring (Schlegel 1966).



(6)

*Poly-β-hydroxybutyric Acid

Most hydrogen bacteria may grow heterotrophically, losing or having a diminished ability to oxidize H_2 if a suitable organic carbon source is present. Under autotrophic conditions an induction of hydrogenase activity occurs. The occurrence of mixotrophic growth with carbon derived from both CO_2 fixation and organic sources has been found to occur in some species and its occurrence in natural environs may be more widespread than expected based on laboratory findings (Bowien and Schlegel 1981; additional reviews in Repaske 1966; Schlegel 1966; Gest 1954).

The second group of aerobic bacteria is able to oxidize H_2 while utilizing N_2 as their sole nitrogen source. These non-symbiotic nitrogen fixers, characterized by the genus Azotobacter, are ubiquitous heterotrophic soil microorganisms. Certain species of the hydrogen bacteria have the dual capacity for autotrophic growth and nitrogen fixation (Bowien and Schlegel 1981). A unidirectional H_2-oxidizing hydrogenase is found in Azotobacter which serves to supply the reducing power for metabolism via oxidative phosphorylation (Hyndman et al 1953). The overall association of hydrogen oxidation with nitrogen fixation is

$$3\ H_2 + N_2 \rightarrow 2\ NH_3 \qquad (7)$$

Under aerobic conditions the ammonia resulting from nitrogen fixation is ultimately oxidized to nitrate in a process known as nitrification. Both ammonia and nitrate nitrogen are readily used by Azotobacter and when present in high quantities may greatly reduce nitrogen fixation (Partridge et al 1980). Considerable fixation of nitrogen by non-symbiotic bacteria may occur on sod land with values as high as 27 kg N per acre being fixed per year (Buckman and Bradley 1967).

Evidence to date, although meagre, suggests that the aerobic nitrogen fixing bacteria may be the group responsible for a substantial portion of observed tritium oxidation. The reduction (~75%) in the atmospheric nitrogen content of the Bruce grassland samples suggested that considerable nitrogen fixation had occurred over 48 h. Additionally, Fallon (1982) found that nitrate added to autoclaved soil which had been reinoculated with soil microflora was not capable of oxidizing elemental tritium. Activity was present, however, in untreated soil or in soil supplemented with an organic carbon source providing nitrate was not added. Fixed nitrogen sources are known to inhibit nitrogen fixation by aerobic non-symbiotic bacteria with a concomittant reduction in hydrogenase activity (Lee and Wilson 1943; Green et al 1953; Green and Wilson 1953; Partridge et al 1980).

Conrad and Seiler (1979) found that three species of hydrogen bacteria, Alcaligenes eutrophus, Paracoccus denitrificans and Xanthobacter autotrophicus, were not capable of oxidizing H_2 at ambient hydrogen levels present in the atmosphere leading them to suggest that other groups of microorganisms, possibly nitrogen fixers, may be responsible. Also the presence of organic carbon in soils may limit the extent of autotrophic existence among the hydrogen bacteria.

In addition to the role which certain metabolic types of bacteria may play in the oxidation of tritium or hydrogen, it has been demonstrated by Conrad and Seiler (1981) that non-metabolic uptake of hydrogen by abiontic soil enzymes may account for 50% or more of total oxidation activity. While oxidation of hydrogen could be stopped by autoclaving, bacteriostatic agents resulted in only partial reduction of uptake activity. Abiontic enzymes may include free extracellular enzymes actively released from bacteria or enzymes bound to dead or dormant cells (Conrad and Seiler 1981; Hiura et al 1979).

Although hydrogenase may activate molecular tritium thereby providing electrons for the reduction of suitable acceptor substances and the energy for further metabolic reactions it may also catalyze an exchange reaction with water that involves no net transfer of electrons (Mortenson and Chen 1974).

$$HT + H_2O \rightleftarrows HTO + H_2 \qquad (8)$$

In the absence of suitable electron acceptors, a possibility with abiontic soil enzymes, a net transfer of HT or T_2 from air into soil water could be mediated by hydrogenase (Fallon 1982).

SUMMARY AND CONCLUSIONS

1. Soils representative of woodlot, pasture and cultivated lands collected from the vicinity of the Bruce and Pickering nuclear power developments were found in laboratory experiments to have the capacity to oxidize elemental tritium. Rates of conversion ranged from 15 to 28% per hour and the process was biologically mediated in soils maintained at a water content of 125% (w/w; ≡ 140% WHC) and incubated at 30°C.

2. Rate of tritium oxidation was slower (3 to 6% per hour) in an artifical soil mixture which may have, in part, resulted from the high water content of the soil under test conditions.

3. Soil moisture content has a pronounced influence on oxidation rate. This could be explained by reduced diffusion of elemental tritium into soils with high water levels due to low solubility of the gas. In Pickering grassland soil incubated at 30°C maximum oxidation ($104\%.h^{-1}$) occurred at a water content of 23% (w/w). At higher water levels the oxidation rate at first decreased, then became constant at 25% of the maximal rate when water content exceeded field capacity (45% w/w). In very dry soil (4% w/w) oxidation was rapid ($77\%.h^{-1}$) but approximately 80% of this activity resulted from abiotic oxidation. In soils with a higher moisture content virtually all oxidation activity was biological.

4. Tritium was oxidized over the entire range of temperatures tested (0 to 51.5°C). Maximum oxidation rates ($\sim 25\%.h^{-1}$) in soils maintained at a water holding capacity of 140% (125% w/w) occurred at temperatures of 20 and 30°C. As temperature approached 50°C oxidation by non-biological processes became increasingly important and accounted for half ($\sim 10\%.h^{-1}$) of the tritium converted at 51.5°C. At lower temperatures oxidation occurred by a biological process.

5. Aging of soil in the laboratory over a three month period resulted in no appreciable change in oxidation activity.

6. Oxygen content of the experimental vessels (1 L capacity) remained unchanged over the 48 h incubation period while the carbon dioxide content increased six fold. The gaseous nitrogen content of the flasks was reduced to 25% of its initial level.

7. Metabolic groups of organisms likely to be responsible for the oxidation of tritium (or hydrogen) were considered. The aerobic free-living nitrogen-fixers, a group of ubiquitous soil bacteria, appear to play a prominent role in the conversion of elemental tritium. Another group of common soil micro-organisms, the hydrogen bacteria, are possibly of some importance and free abiotic soil enzymes may also be involved.

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APPENDIX I

WEIGHT LOSS OF SOIL* DURING 24 HOUR DRYING PERIOD AT 70°C

Drying Time (h)	Weight of Soil (g) Replicate 1	Replicate 2
0	97.1	94.3
1	82.2	79.1
4	78.5	75.9
6	78.4	75.8
24	78.3	75.7

* Bruce Pasture Grassland

APPENDIX II

SCHEDULE FOR TRITIUM OXIDATION EXPERIMENTS

Phase I - Soil Comparison	Date	Injected Tritium Level (μCi)
Used Potting Soil	Nov 16/81	1.65
Pickering Woodlot	Nov 23/81	1.65
Pickering Cultivated	Nov 25/81	1.65
Pickering Grassland	Nov 30/81	1.65
Bruce Woodlot	Dec 2/81	1.65
Bruce Cultivated	Dec 7/81	1.65
Bruce Grassland	Dec 9/81	1.65
New Potting Soil	Dec 16/81	1.65
Phase II - Soil Moisture Content		
5% WHC	Jun 23/82	9.75
25% (1)	Jan 13/82	1.65
25% (2)	May 12/82	9.75
50% (1)	Jan 27/82	1.65
50% (2)	May 18/82	9.75
75% (1)	Jan 19/82	1.65
75% (2)	May 11/82	9.75
100%	Jan 18/82	1.65
140%	Jan 11/82	1.65
Phase III - Soil Temperature		
0°C	May 31/82	9.75
10.0	Jun 2/82	9.75
20.0	Feb 10/82	1.65
30.0	Feb 24/82	1.65
40.0	Jun 7/82	9.75
50.0	May 25/82	9.75
50.0	May 27/82	9.75*
51.5	Jun 21/82	9.75

* Sampled at 0 and 48 hours

APPENDIX III

EXPERIMENTAL DATA FOR SOIL COMPARISONS
(Values given as 10^6 DPM's per Flask)

Soil	Time	Replicates 1	2	3	Water Content of Flask (g)
Bruce Cultivated	0	0.0019	0.0011	0.0015	18.33
	1	1.0080	1.0139	1.1567	18.33
	2	1.3028	1.2215	1.3650	18.33
	8	2.4594	2.0235*	2.4622	18.33
	20	3.2036	3.1614	3.1250	18.33
	26	3.2683	3.2454	3.1675	18.33
	48	3.2845	3.2813	3.1794	18.33
Bruce Grassland	0	0.0016	0.0013	0.0021	15.95
	1	0.5102	0.4239	0.4617	15.95
	2	0.9412	1.3379	0.9150	15.95
	8		2.4482	2.4916	15.95
	20	3.2640	2.9135	3.2373	15.95
	26	3.4962	3.2054	3.3512	15.95
	48	3.2133	3.2064	3.1019	15.95
Bruce Woodlot	0	0.0105	0.0135	0.0110	36.16
	1	0.5352	0.5341	0.5276	36.16
	2	0.9345	0.9703	0.9087	36.16
	8	2.8808	2.8182	2.6208	36.16
	20	3.0819	3.2565	3.2018	36.16
	26	3.2594	3.2495	3.2925	36.16
	48	3.3139	3.1839	3.2367	36.16
New Potting Soil	0	0.0002	0.0000	0.0016	207.60
	1	0.2001	0.2363	0.1913	207.60
	2	0.3613	0.3986	0.3024	207.60
	8	1.0521	1.0789	0.9838	207.60
	20	2.3159	2.1246	2.2380	207.60
	26	2.4692	2.5007	2.3182	207.60
	48	2.9587	3.0568	3.0481	207.60
Pickering Cultivated	0	0.0226	0.0415	0.0349	15.95
	1	0.6783	0.5510	0.5619	15.95
	2	0.9685	0.8969	1.3320	15.95
	8	2.2826	2.2652	2.4184	15.95
	21	3.4513	3.5102	3.3230	15.95
	26	3.5298	3.4861	3.5658	15.95
	48	3.3489	3.6616	3.7101	15.95
Pickering Grassland	0	0.0313	0.0204	0.0225	25.02
	1	0.8072	0.6942	0.9992	25.02
	2	1.0642	1.4231	1.3008	25.02
	8	5.5163*	1.4972*	2.9921	25.02
	20	3.3502	4.0589	3.4753	25.02
	26	4.8396	3.5750	4.0321	25.02
	48	4.6708	4.2014	3.2963	25.02
Pickering Woodlot	0	0.0103	0.0082	0.0152	28.49
	1	0.6941	0.7014	0.6687	28.49
	2	1.3758	1.2776	1.9007	28.49
	8	3.7410*	2.8528	2.9560	28.49
	20	3.4498	3.4396	3.4726	28.49
	26	3.5227	3.4116	3.3502	28.49
	48	3.3797	3.4653	3.5304	28.49
Used Potting Soil	0	0.0493	0.0825	0.0818	207.60
	1	0.1919	0.1787	0.1663	207.60
	2	0.3842	0.3950	0.4058	207.60
	8	0.8221	0.8978	0.7099	207.60
	20	1.9631	1.7892	1.7193	207.60
	26	2.1581	1.8903	1.8971	207.60
	48	3.0028	2.8784	2.8869	207.60

* Values indicated are possible outliers from model fitting

APPENDIX IV

NON-LINEAR LEAST SQUARES SUMMARY STATISTICS FOR TRITIUM OXIDATION RATES IN DIFFERENT SOILS

Soil	Source	DF	Sum of Squares	Mean Square	Parameter	Estimate *	Asymptotic Std Error
Bruce Cultivated	Regression	2	116.45707361	58.22853680			
	Residual	19	1.11711263	0.05879540			
	Uncorrected Total	21	117.57418624		Asymptote	3.15909020	0.08197489
					Oxidation Rate	0.23756713	0.02685634
	(Corrected Total)	20	29.79735110				
Bruce Grassland	Regression	2	109.60034673	54.80017336			
	Residual	18	0.34632971	0.01924054			
	Uncorrected Total	20	109.94667644		Asymptote	3.25578047	0.05084767
					Oxidation Rate	0.18275222	0.01267881
	(Corrected Total)	19	35.74253449				
Bruce Woodlot	Regression	2	120.35543663	60.17771831			
	Residual	19	0.21912358	0.01153282			
	Uncorrected Total	21	120.57456021		Asymptote	3.27972977	0.03815135
					Oxidation Rate	0.19970044	0.00980619
	(Corrected Total)	20	37.20821815				
New Potting Soil	Regression	2	63.64156677	31.82078338			
	Residual	19	0.10952889	0.00576468			
	Uncorrected Total	21	63.75109566		Asymptote	3.30146340	0.07711606
					Oxidation Rate	0.05228564	0.00274350
	(Corrected Total)	20	26.85176667				
Pickering Cultivated	Regression	2	131.36686906	65.68343453			
	Residual	19	0.41313916	0.02174417			
	Uncorrected Total	21	131.78000822		Asymptote	3.57613072	0.05903757
					Oxidation Rate	0.14653153	0.00948192
	(Corrected Total)	20	41.08910513				
Pickering Grassland	Regression	2	180.27106911	90.13553455			
	Residual	19	10.90632104	0.57401690			
	Uncorrected Total	21	191.17739015		Asymptote	3.99062308	0.26526339
					Oxidation Rate	0.20914269	0.05924131
	(Corrected Total)	20	63.06765938				
Pickering Woodlot	Regression	2	145.55804072	72.77902036			
	Residual	19	0.82886501	0.04362447			
	Uncorrected Total	21	146.38690573		Asymptote	3.47090970	0.06015721
					Oxidation Rate	0.27813346	0.02424549
	(Corrected Total)	20	40.19367451				
Used Potting Soil	Regression	2	49.77217408	24.88608704			
	Residual	19	0.27077115	0.01425111			
	Uncorrected Total	21	50.04294524		Asymptote	3.66067444	0.24181441
					Oxidation Rate	0.03257025	0.00378802
	(Corrected Total)	20	21.34125450				

* Asymptote values given as 10^6 DPM's per flask; Oxidation Rates given as fractional conversion per hour

APPENDIX V

Estimates of the Variance in Measurements by Soil Type

Soil Type	SS	d.f.	S^2	S	3S	
BRCULTIV	0.16785	12	0.01399	0.11827	0.35481	
BRGRASLD	0.24322	11	0.02211	0.14870	0.44610	
BRWOODLT	0.06429	12	0.00536	0.07319	0.21958	
NPOTTING	0.05409	12	0.00451	0.06714	0.20142	
PKCULTIV	0.23122	12	0.01927	0.13881	0.41643	
PKGRASLD	10.44954	12	0.87080	0.93316	2.79949	← Note large variance for this soil type
PKWOODLT	0.72425	12	0.06035	0.24567	0.73701	
UPOTTING	0.10627	12	0.00886	0.09411	0.28232	
PKGRASLD*	2.19646	10	0.21965	0.46867	1.40599	
Pooled*	3.78776	93	0.04073	0.20181	0.60544	

SS = corrected sum of squares

df = degrees of freedom for SS

S^2 = estimated variance

S = estimated standard deviation

3S = approximate 99% confidence band for |residual|

This was estimated from the variance of the replications about the means of the replications.

- time = 0 was not used
- all other data points used

PKGRASLD* - the calculations were repeated for soil type PKGRASLD but outliers were deleted.

Pooled* - uses the corrected variance for PKGRASLD

APPENDIX VI

EXPERIMENTAL DATA FOR WATER CONTENT PICKERING PASTURE-GRASSLAND SOIL

Water Content (% WHC)	Time (h)	Replicates 1	2	3
5	0.00	0.001	0.001	0.002
	0.25	0.077	0.121	0.101
	0.50	0.121	0.144	
	1.00	0.313	0.375	
	2.00	0.477	0.436	
	4.00	0.566	0.520	
	20.00	0.566	0.576	0.540
	24.00	0.544	0.578	0.577
	48.00	0.589	0.612	0.582
25 (1)	0.00	0.002	0.004	0.003
	1.00	0.567	0.596	0.628
	2.00	0.587	0.589	0.612
	4.00	0.616	0.533	0.622
	8.00	0.603	0.582	0.633
	24.00	0.615	0.608	0.619
	48.00	0.606	0.626	0.616
25 (2)	0.00	0.015	0.016	0.016
	0.25	0.019	0.127	0.015
	0.50	1.037	0.476	0.729
	1.00	1.295	1.185	1.027
	2.00	1.207	1.278	1.464
	4.00	1.544	1.634	1.618
	24.00	1.501	1.433	1.521
	48.00	1.678	1.748	0.165*
50 (1)	0.00	0.001	0.001	0.001
	1.00	0.088	0.093	0.096
	2.00	0.133	0.146	0.157
	4.00	0.205	0.133*	0.125*
	8.00	0.265	0.261	0.274
	24.00	0.294	0.124*	0.136*
	48.00	0.287	0.292	0.285
50 (2)	0.00	0.006	0.005	0.005
	1.00	0.628	0.584	0.597
	2.00	0.958	0.026*	0.343*
	4.00	1.593	1.461	1.606
	8.00	1.676	1.710	1.644
	24.00	1.932	1.923	1.893
	48.00	1.937	1.925	1.943
75 (1)	0.00	0.000	0.000	0.000
	1.00	0.026	0.021	0.016
	2.00	0.056	0.052	0.053
	4.00	0.063	0.068	0.053
	8.00	0.091	0.105	0.096
	24.00	0.170*	0.159*	0.152*
	48.00	0.104*	0.094*	0.125*
75 (2)	0.00	0.000	0.001	0.000
	1.00	0.066	0.065	0.047
	2.00	0.189	0.148	0.207
	4.00	0.462	0.349	0.426
	8.00	0.904	0.971	0.791
	24.00	1.057	1.081	1.032
	48.00	1.107	1.197	1.166
100	0.00	0.000	0.000	0.000
	1.00	0.035	0.034	0.036
	2.00	0.065	0.060	0.061
	4.00	0.102	0.098	0.100
	8.00	0.142	0.142	0.137
	24.00	0.168	0.165	0.167
	48.00	0.171	0.173	0.169
140	0.00	0.000	0.000	0.000
	1.00	0.024	0.020	0.022
	2.00	0.042	0.038	0.044
	4.00	0.068	0.070	0.070
	8.00	0.101	0.099	0.098
	24.00	0.120	0.119	0.119
	48.00	0.119	0.116	0.117

* Values indicated are possible outliers from model fitting

APPENDIX VII

NON-LINEAR LEAST SQUARES SUMMARY STATISTICS FOR TRITIUM OXIDATION RATES AT VARIOUS SOIL WATER CONTENTS; PICKERING PASTURE-GRASSLAND SOIL

Water Content (% WHC)	Source	DF	Sum Of Squares	Mean Square	Parameter	Estimate*	Asymptotic Std Error
5	Regression	2	4.26274055	2.13137027			
	Residual	21	0.01689650	0.00080460	Asymptote	0.57411138	0.00890721
	Uncorrected Total	23	4.27963705		Oxidation Rate	0.77141386	0.05054209
	(Corrected Total)	22	1.19784035				
25(1)	Regression	2	6.54557865	3.27278932			
	Residual	19	0.01026349	0.00054018	Asymptote	0.60427919	0.00603065
	Uncorrected Total	21	6.55584213		Oxidation Rate	4.39568966	1.97785231
	(Corrected Total)	20	0.93603781				
25(2)	Regression	2	30.66996561	15.33498280			
	Residual	21	0.71382591	0.03399171	Asymptote	1.58929813	0.06581202
	Uncorrected Total	23	31.38379151		Oxidation Rate	1.04831725	0.14925700
	(Corrected Total)	22	9.21019950				
50(1)	Regression	2	0.67876932	0.33938466			
	Residual	15	0.00080085	0.00005339	Asymptote	0.28677938	0.00316908
	Uncorrected Total	17	0.67957017		Oxidation Rate	0.35212350	0.01289574
	(Corrected Total)	16	0.19254263				
50(2)	Regression	2	39.85124993	19.92562497			
	Residual	17	0.09077862	0.00533992	Asymptote	1.88967060	0.02672522
	Uncorrected Total	19	39.94202855		Oxidation Rate	0.38309820	0.02130825
	(Corrected Total)	18	9.56325313				
75(1)	Regression	2	0.04922722	0.02461361			
	Residual	13	0.00086886	0.00006684	Asymptote	0.10984668	0.01094416
	Uncorrected Total	15	0.05009608		Oxidation Rate	0.25178129	0.05028088
	(Corrected Total)	14	0.01728315				
75(2)	Regression	2	10.23536421	5.11768211			
	Residual	19	0.14765689	0.00777142	Asymptote	1.15153580	0.04136308
	Uncorrected Total	21	10.38302110		Oxidation Rate	0.13155371	0.01374691
	(Corrected Total)	20	4.33821422				
100	Regression	2	0.27563937	0.13781969			
	Residual	19	0.00009299	0.00000489	Asymptote	0.16902050	0.00089463
	Uncorrected Total	21	0.27573236		Oxidation Rate	0.22579474	0.00368827
	(Corrected Total)	20	0.07996824				
140	Regression	2	0.13470694	0.06735347			
	Residual	19	0.00005737	0.00000302	Asymptote	0.11894233	0.00070750
	Uncorrected Total	21	0.13476431		Oxidation Rate	0.21832656	0.00398597
	(Corrected Total)	20	0.04052054				

* Asymptote values given as 10^6 DPM's per flask; Oxidation rate given as fractional conversion per hour

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APPENDIX VIII

EXPERIMENTAL DATA FOR TEMPERATURE PICKERING PASTURE-GRASSLAND SOIL

Temperature (°C)	Time (h)	Replicates 1	2	3
0	0.00	0.001	0.001	0.001
	1.00	0.073	0.060	0.045
	2.00	0.140	0.097	0.096
	4.00	0.251	0.220	0.246
	8.00	0.377	0.327	0.354
	24.00	0.652		0.630
	48.00	0.674	0.640	0.666
10.0	0.00	0.001	0.000	0.000
	1.00	0.067	0.077	0.035
	2.00	0.167	0.070	0.155
	4.00	0.246	0.091	0.290
	8.00	0.381	0.370	0.384
	24.00	0.525	0.269*	0.678
	48.00	0.607	0.660	0.661
20.0	0.00	0.000	0.000	0.000
	1.00	0.012	0.008	0.010
	2.00	0.021	0.019	0.015
	4.00	0.028	0.027	0.025
	8.00	0.055	0.059	0.027
	24.00	0.030	0.040	0.050
	48.00	0.051	0.048	0.047
30.0	0.00	0.000	0.000	0.000
	1.00	0.020	0.020	0.018
	2.00	0.036	0.034	0.031
	4.00	0.050	0.052	0.051
	8.00	0.078	0.081	0.064
	24.00	0.088	0.086	0.086
	48.00	0.087	0.083	0.090
40.0	0.00	0.000	0.001	0.002
	1.00	0.018	0.119	0.094
	2.00	0.014	0.155	0.014
	4.00	0.323	0.385	0.327
	8.00	0.522	0.651	0.516
	24.00	0.645	0.724	0.648
	48.00	0.643	0.846	0.671
51.5	0.00	0.000	0.000	0.000
	1.00	0.046	0.043	0.028
	2.00	0.098	0.081	0.063
	4.00	0.130	0.105	0.098
	6.50	0.157	0.200	0.210
	22.00	0.217	0.220	0.216
	24.00	0.207	0.234	0.213
	48.00	0.255	0.289	0.218

* Value indicated is a possible outlier from model fitting

APPENDIX IX

NON-LINEAR LEAST SQUARES SUMMARY STATISTICS FOR TRITIUM OXIDATION RATES AT VARIOUS SOIL TEMPERATURES PICKERING PASTURE-GRASSLAND SOIL

Temperature (°C)	Source	DF	Sum of Squares	Mean Square	Parameter	Estimate*	Asymptotic Std Error
0	Regression	2	2.71653308	1.35826654	Asymptote	0.67643292	0.01201684
	Residual	18	0.00830518	0.00046140	Oxidation Rate	0.09903068	0.00477759
	Uncorrected Total	20	2.72483826				
	(Corrected Total)	19	1.18339013				
10.0	Regression	2	2.56051159	1.28025580	Asymptote	0.60147776	0.04359665
	Residual	19	0.13789064	0.00725740	Oxidation Rate	0.10882642	0.02250080
	Uncorrected Total	21	2.69840223				
	(Corrected Total)	20	1.13298745				
20.0	Regression	2	0.02170503	0.01085251	Asymptote	0.04587514	0.00303102
	Residual	19	0.00114220	0.00006012	Oxidation Rate	0.26835506	0.05652521
	Uncorrected Total	21	0.02284723				
	(Corrected Total)	20	0.00721856				
30.0	Regression	2	0.07407317	0.03703658	Asymptote	0.08685140	0.00134934
	Residual	19	0.00021538	0.00001134	Oxidation Rate	0.23620286	0.01141337
	Uncorrected Total	21	0.07428854				
	(Corrected Total)	20	0.02115458				
40.0	Regression	2	4.18532564	2.09266282	Asymptote	0.71857620	0.03636789
	Residual	19	0.12385475	0.00651867	Oxidation Rate	0.14726552	0.02188662
	Uncorrected Total	21	4.30918039				
	(Corrected Total)	20	1.75812181				
51.5	Regression	2	0.64157519	0.32078759	Asymptote	0.23325783	0.00762723
	Residual	22	0.01031956	0.00046907	Oxidation Rate	0.19962394	0.02163521
	Uncorrected Total	24	0.65189475				
	(Corrected Total)	23	0.19039017				

* Asymptote values given as 10^6 DPM's per flask; Oxidation rates given as fractional conversion per hour

APPENDIX X

EXPERIMENTAL DATA FOR HEAT STERILIZED SAMPLES
(Values Given as 10^6DPM's per Flask)

Soil/Treatment	Time (h)	Replicates 1	2	3
Phase I - Soil Comparison				
Bruce Cultivated	0	0.0000	0.0000	0.0000
	48	0.0030	0.0028	0.0028
Bruce Grassland	0	0.0000	0.0000	0.0000
	48	0.0020	0.0019	0.0034
Bruce Woodlot	0	0.0000	0.0000	0.0000
	48	0.0030	0.0030	0.0033
New Potting Soil	0	0.0000	0.0000	0.0000
	48	0.0000	0.0000	0.0000
Pickering Cultivated	0	0.0001	0.0000	0.0000
	48	0.0000	0.0000	0.0001
Pickering Grassland	0	0.0000	0.0000	0.0000
	48	0.0039	0.0040	0.0035
Pickering Woodlot	0	0.0000	0.0000	0.0000
	48	0.0001	0.0001	0.0001
Used Potting Soil	0	0.0000	0.0000	0.0000
	48	0.0003	0.0002	0.0006
Phase II - Soil Moisture Content*				
5% WHC	0	0.0002	0.0001	0.0001
	48	0.5394	0.4444	0.4136
25% (1)	0	0.0003	0.0000	0.0004
	48	0.0006	0.0006	0.0004
25% (2)	0	0.0022	0.0026	0.0019
	48	0.0200	0.0109	0.0104
50% (1)	0	0.0001	0.0001	0.0001
	48	0.0004	0.0003	0.0004
50% (2)	0	0.0012	0.0012	0.0010
	48	0.0016	0.0017	0.0018
75% (1)	0	0.0002	0.0002	-
	48	0.0002	0.0002	-
75% (2)	0	0.0000	0.0009	0.0000
	48	0.0008	0.0004	0.0004
100%	0	0.0000	0.0000	0.0000
	48	0.0001	0.0001	0.0001
140%	0	0.0000	0.0000	0.0000
	48	0.0001	0.0001	0.0000
Phase III - Soil Temperature*				
0°C	0	0.0001	0.0001	0.0001
	48	0.0003	0.0003	0.0003
10.0	0	0.0001	0.0001	0.0000
	48	0.0002	0.0001	0.0002
20.0	0	0.0001	0.0001	0.0001
	48	0.0001	0.0001	0.0001
30.0	0	0.0000	0.0000	0.0000
	48	0.0001	0.0001	0.0002
40.0	0	0.0001	0.0001	0.0001
	48	0.0006	0.0007	-
50.0	0	0.0035	0.0031	0.9930
	48	0.0530	0.1043	0.0538
51.5	0	0.0000	0.0000	0.0000
	48	0.1331	0.1915	-

* Pickering Pasture - Grassland Soil

POOR

QUALITY

ORIGINAL